AMENDMENT(S) TO THE SPECIFICATION

Please amend the specification as follows:

Please insert a paragraph beginning at page 1, line 2:

CROSS REFERENCE TO RELATED APPLICATIONS

The present application is a 35 U.S.C. § 371 national phase conversion of PCT/EP2004/002170 filed 03 March 2004, which claims priority of German Application No. 103 10 261.2 filed on 05 March 2003.

The PCT International Application was published in the German language.

On page 1, after the Cross Reference To Related Applications, delete "Description" and insert:

Field of the Invention

On page 1, please replace the first paragraph beginning, "The present invention relates to..." with the following rewritten paragraph:

The present invention relates to methods for identifying and/or detecting T-cell epitopes of [[the]] protein ~~antigen~~ antigens, to methods for preparing peptide vaccines against a protein antigen, to methods for controlling the quality of receptor/ligand complexes and/or components thereof, to methods for preparing nanoparticles having at least one immobilized receptor unit or an immobilized receptor, to methods for preparing nanoparticles having immobilized peptide-presenting MHC molecules, to methods for enriching and/or isolating specific $CD4^+$-T- or $CD8^+$-T-lymphocytes from peripheral blood mononuclear cells, to methods for priming and/or restimulating a $CD4^+$-T- or $CD8^+$-T-lymphocyte reaction in vitro, to nanoparticles having an immobilized receptor unit, in particular an immobilized chain of an MHC molecule, to nanoparticles having an immobilized receptor, in particular an immobilized MHC molecule, to nanoparticles having an immobilized

peptide-presenting MHC receptor, to a peptide vaccine, to a kit for identifying and/or detecting T-cell epitopes of a protein antigen, and to the use of the nanoparticles for identifying and/or detecting T-cell epitopes, for preparing peptide vaccines, for enriching and/or isolating specific T-lymphocytes and for priming a $CD4^+$-T- or $CD8^+$-T-lymphocyte reaction in vitro.

On page 2, before the first paragraph beginning with, "The health of an animal ...", please insert:

Background of the Invention

On page 6, between lines 2-3, before the paragraph beginning with, "The technical object of the present invention...", please insert:

Summary of the Invention

On page 6, please replace the paragraph beginning, "The technical object of the present invention...", with the following rewritten paragraph:

The technical object of the present invention is to provide an improved method for screening potential T-cell epitopes, which method allows 2 simultaneous and rapid ~~examination~~ examinations of a large number of peptide sequences, for example sequences which have already been determined as potential binding partners for specific MHC molecules using computer algorithms, for their capability of binding to specific MHC molecules.

On page 9, between lines 8-9, before the paragraph beginning with ,"In the context of the present invention... ", please insert:

Brief Description of the Figures

FIG. 1 schematically illustrates a preferred method according to the invention for identifying and/or detecting T-cell epitopes;

FIG. 2 illustrates mass spectrograms, obtained by MALDI mass spectrometry, of nanoparticles having immobilized peptide - presenting HLA complexes, wherein:

FIG. 2.1 provides a spectrogram of a peptide mixture of equimolar amounts of five peptides; and

FIG 2.2 provides a spectrogram of two peptides identified, after selection, as binding; and

FIG. 3 provides a MALDI mass spectrograph illustrating all of the molecular components of the HLA-A2-EBNA-6 complex immobilized on SAV nanoparticles.

Detailed Description of the Invention

On page 46, please replace the paragraph beginning, "Figure 1 shows, in schematic form, ...", with the following rewritten paragraph:

Figure 1 shows, in schematic form, a preferred embodiment of the method according to the invention for identifying and/or detecting T-cell epitopes, where a peptide-presenting HLA-A2 complex prepared in solution is immobilized on nanoparticles. The nanoparticles having the complex are then treated with an acidic stripping buffer, resulting in the removal of the EBV-EBNA-6 peptide (positions 284-293, LLDFVRFMGV (SEQ ID No. 1)) and β-2-microglobulin (β_2-m). The nanoparticles prepared in this manner having the immobilized HLA chain are then used for carrying

out a competitive binding reaction using a peptide population in the presence of β_2-m, where the peptide(s) having affinity binds/bind to HLA and β_2-m, resulting in the formation, on the nanoparticle surface, of an HLA complex presenting this/these peptide(s). Following removal of the unbound peptides and excess β_2-m, the nanoparticles having the immobilized peptide-presenting complex are subjected to analysis by MALDI mass spectrometry.

On page 47, between lines 8-9, please insert:

Examples

The following Examples are provided only for the purpose of illustrating the invention and are not to be constrained as limiting the invention in any way.

On pages 47-48, please replace the paragraph beginning, "Soluble HLA-A*0201 peptide tetramers...", with the following rewritten paragraph:

Soluble HLA-A*0201 peptide tetramers were synthesized as described by Altman et al., Science, 274 (1996), 94-96. Recombinant heavy HLA-A*0201 chains (positions 1-276) in soluble form and β-2-microglobulin (β_2-m) were expressed separately in Escherichia coli cells which had been transformed using appropriate expression plasmids. The 3'-terminus of the extracellular domains of the heavy HLA-A*0201 chain were modified using a BirA biotinylation sequence. The Escherichia coli cells which had been transformed with the appropriate expression plasmids coding for the HLA-A*0201 chain or β_2-m were cultivated until they reached the mid-log growth phase. They were then induced using 0.5 isopropyl β-galactosidase. After further cultivation and expression of the recombinant proteins, the Escherichia coli cells were harvested and purified. After cell disruption, the inclusion bodies present in the cells were isolated, purified and solubilized in 8 M urea, pH 8.0. The heavy HLA-A*0201 chain and β_2-m were diluted in 100 mM Tris, 2 mM EDTA,

400 mM L-arginine, 5 mM reduced glutathione and 0.5 mM oxidized glutathione, and 10 μM of the peptide LLDFVRFMGV (SEQ ID No. 1) (EBV EBNA-6, positions 284-293) were added. The mixture was then incubated with stirring at 10°C for 48 hours. The folded 48 kDa complexes (α-chain: about 35 kDa, β_2-m: about 12 kDa, peptide: about 1 kDa) were concentrated by ultrafiltration using a membrane having a retention capacity of 10 kDa (Millipore, Bedford, USA) and purified by HPSEC using a Superdex G75 HiLoad 26/60 column (Amersham Pharmacia Biotech, Upsala, Sweden) and 150 mM NaCl, 20 mM Tris-HCl, pH 7.8, as elution buffer. Following gel filtration, the purified monomers were biotinylated using a biotin ligase (BirA; Avidity, Denver, USA) and repurified by HPSEC. The complex was then adjusted to a concentration of 1 mg/ml by ultrafiltration.

On pages 49-50, please replace the paragraph beginning, "All washing steps of the nanoparticles...", with the following rewritten paragraph:

All washing steps of the nanoparticles were carried out by centrifuging for 10 minutes at 15 000 x g at 20°C in a temperature-controlled centrifuge in 1.5 ml reaction vessels and by resuspending the beads using a micropipette. 55 μg of SAV nanoparticles and 3.5 μg of the soluble HLA-A2 complex comprising the peptide LLDFVRFMGV (SEQ. ID No. 1) (EBV EBNA-6, positions 284-293) were suspended in 20 μl of PBS. For 2 hours, the mixture was incubated in a horizontal shaker at room temperature to prevent sedimentation. After 10 minutes of centrifugation at 20°C, the supernatant was discarded and the nanoparticles were washed with 50 μl of water. To release β_2-m molecules and the peptide LLDFVRFMGV (SEQ ID No. 1) comprised in the complex, the beads were incubated for 90 seconds in 150 μl of stripping buffer (50 mM sodium citrate, pH 3.0) and, after centrifugation, washed with 150 μl of water. The beads were then resuspended using 30 μl of PBS containing 1.2 μg of β_2-m molecules (Sigma, Munich, Germany) and a peptide mixture.

The mixture consisted of a total of 5 peptides in an amount of in each case 0.072 µg. The 5 peptides had the sequences ILMEHIHKL (SEQ ID No. 2), DQKDHAVF (SEQ ID No. 3), ALSDHHIYL (SEQ ID No. 4), VITLVYEK (SEQ ID No. 5) and SNEEPPPPY (SEQ ID No. 6). After four hours of incubation at 37°C, the nanoparticles were pelleted by centrifugation and, after removal of the supernatant, washed with 50 ml of PBS buffer and then 50 µl of water. After the final centrifugation, the nanoparticles were resuspended in 0.1% water/TFA (v/v) and transferred to a MALDI target. Analysis was carried out using a Voyager DE-STR mass spectrometer (Applied Biosystems Foster City, USA) in positive ion reflection mode. Solutions comprising proteins and peptides were mixed on the target with an identical matrix volume using a 1:20 dilution of saturated α-cyano-4-hydroxycinnamic acid or sinapinic acid in 30% acetonitrile/0.3% TFA (v/v). All MALDI spectra were calibrated externally using a standard peptide mixture.

On page 51, please replace the paragraph beginning, "Complete complexes immobilized via biotin...", with the following rewritten paragraph:

Complete complexes immobilized via biotin on the SAV particles (SAV nanobeads) were visualized by MALDI mass spectrometry, the corresponding mass signals for the biotinylated HLA-A2 α-chain being 34379 Da, that for β_2-m molecules being 11727 Da, that for the streptavidin monomer being 12907 Da and that for the bound peptide LLDFVRFMGV (SEQ ID No. 1) being 1196.63 Da (Figure 3). Using the MALDI-TOF method, it was thus possible to check both the correct properties of the HLA-A2 complex on the one hand and the effectiveness of the method for immobilizing the biotinylated complex on the SAV nanoparticles.

On pages 51-52, please replace the paragraph beginning, "Figure 2 shows the MALDI Spectra of a peptide...", with the following rewritten paragraph:

Figure 2 shows the MALDI spectra of a peptide mixture comprising two HLA-A2 peptides which bind and three peptides which do not bind, each peptide being present in an amount of about 70 pmol. The predicted binding of the peptides was determined using the SYFPEITHI program, where, at very strong binding, a score of 32 was determined for the peptide ILMEHIHKL (SEQ ID No. 2), at a very strong binding, a score of 23 was determined for the peptide ALSDHHIYL (SEQ ID No. 4) and for the three nonbinding proteins a score of 0 was determined. The different signal intensities of the respective peptides in the mixture used are the result of different ionization capacities. The identity of the observed peaks was confirmed by MALDI-PSD sequencing. Following selection of the peptides having HLA receptors, after treatment, i.e. washing with PBS buffer, only the signals for the binding peptides remained. The fact that no signal could be detected for the nonbinding proteins shows that there are no unspecific interactions. The spectra show the monoisotopic mass for each peptide in protonated form ($[M+H]^+$) and the monoisotope in sodium form ($[M+Na]^+$).

Please add pages 52A-52B in the specification (before the claims).

On page 53, please delete "Claims" and insert:

What is claimed is: